American Journal of Medical Genetics 92:178–183 (2000)

Anticipation in Inflammatory Bowel Disease: A Phenomenon Caused by an Accumulation of Confounders

Jochen Hampe,[1] Kerstin Heymann,[1,2] Wolfgang Kruis,[3] Andreas Raedler,[4] Ullrich R. Fölsch,[1] and Stefan Schreiber[1,2]*

[1]*First Department of Medicine, Christian-Albrechts University, Kiel, Germany*
[2]*Fourth Department of Medicine, Charité University Hospital, Berlin, Germany*
[3]*Evangelisches Krankenhaus Kalk, Köln, Germany*
[4]*Tabea IBD Center, Hamburg, Germany*

Inflammatory bowel disease (IBD) has a definite genetic component as documented by epidemiological and linkage evidence. It shows an earlier onset of disease in children of affected patients than in their parents. This has lead to speculations about genetic anticipation in this disorder. 2,007 IBD patients with sporadic disease and 472 multiplex familial cases (including 103 affected parents and 99 children of affected patients) were evaluated with a multi-item questionnaire as part of a study of inflammatory bowel disease genetics. The Mann-Whitney U-test and the general linear model were used for analysis. Clinical characteristics such as presence of fistulae, stenoses, extraintestinal manifestations, and other parameters, which are related to the severity of the disease, were found to be similar between familial and sporadic cases of IBD (corrected $P \geq 0.31$ for all tests). The mean-age-of onset in children of affected patients was 19.4 years earlier than in their parents. However, the age of the parental cohort was significantly higher (27 years) and the diagnostic interval also longer (1.7 years). If these confounders are corrected in a general linear model, no significant difference is evident for the age-of-onset between the groups ($P \geq 0.52$). There is no evidence for genetic anticipation in inflammatory bowel disease. The absence of genetic anticipation is consistent with the clinical similarity of familial and sporadic inflammatory bowel disease. This finding justifies the primary genetic analysis of familial disease under the assumption that their genetic background will be representative for all presentations of IBD. Am. J. Med. Genet. 92:178–183, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: inflammatory bowel disease; genetic anticipation; genetic epidemiology; Crohns disease; ulcerative colitis

INTRODUCTION

Genetic factors play an important role in the cause of inflammatory bowel disease as documented by epidemiological and linkage evidence [Cho et al., 1998; Hampe et al., 1999; Hugot et al., 1994; Orholm et al., 1991; Satsangi et al., 1996b]. Inflammatory bowel disease (IBD) is characterized by a chronic relapsing intestinal inflammation leading to abdominal pain, diarrhea, weight loss, and possible complications such as intestinal stenoses, fistulae, and colon cancer. IBD is subdivided into Crohns disease and ulcerative colitis phenotypes, based on clinical and histological characteristics [Podolsky, 1991].

An earlier age-of-onset of disease in children of affected individuals with inflammatory bowel disease has been observed consistently [Grandbastien et al., 1998; Lee and Lennard Jones, 1996; Polito et al., 1996; Satsangi et al., 1998]. The mean differences in age-of-onset between affected children and their affected parents ranged from 10 to 16 years in different cohorts. In the interpretation of these findings, genetic anticipation was suggested as the mechanism to trigger the earlier age-of-onset in these children [Grandbastien et al., 1998; Lee and Lennard Jones, 1996; Polito et al., 1996; Satsangi et al., 1998].

An attractive molecular hypothesis to explain anticipation would be the expansion of unstable trinucleotide repeats in disease-causing genes. This mechanism is

Grant sponsor: the "Deutsche Forschungsgemeinschaft"; Grant numbers: Schr 512/4-1, SFB15; Grant sponsor: "Kompetenznetzwerk" grant from the BmBF; Grant number: 01 GI9984; Grant sponsor: Training and Mobility of Researchers grant, the European Commission; Grant number: ERB-4061-P2-97-0384; Grant sponsor: MFG.

*Correspondence to: Stefan Schreiber, Christian-Albrechts-University, 1st Department of Medicine, Schittenhelmstr. 12, D-24105 Kiel, Germany. E-mail: s.schreiber@mucosa.de

Received 7 May 1999; Accepted 4 February 2000

responsible for a number of hereditary neurological disorders including the fragile-X-syndrome, Huntingtons chorea, and others [Ashley and Warren, 1995; Duyao et al., 1993; The Huntingtons Disease Collaborative Research Group, 1993]. The presence of these repeats would offer an elegant direct approach to the molecular identification of potential susceptibility genes. However, inflammatory bowel disease is a complex polygenic disorder and does not follow a clear mode of inheritance [Küster et al., 1989; Orholm et al., 1993]. In polygenic diseases, the verification of genetic anticipation is much more difficult than in Mendelian disorders because it may be confounded by a number of intrinsic and extrinsic factors [McInnis, 1996; Penrose, 1948; Vieland and Hodge, 1995].

The existence of genetic anticipation would also imply a difference in the characteristics of familial and sporadic IBD. True genetic anticipation should not only increase the risk to develop disease in cases with a parental family history but should also have an influence on the disease characteristics (i.e., severity). Several studies indicated a striking similarity of the clinical and epidemiological properties between familial and sporadic IBD [Lee and Lennard Jones, 1996]. However, it has been also argued that cohorts in these studies were too small to detect differences.

If a significant difference between familial and sporadic disease exists, the commonly used approach of identifying susceptibility genes in familial disease and applying the results to sporadic cases would loose its justification. It would have to be assumed that the molecular cause of familial disease (i.e., anticipation causing genetic defects) is different from sporadic disease (e.g., spontaneous mutations).

We used a large German sample of familial and sporadic IBD patients to examine the relationship between both forms of disease as well as familial anticipation. We investigated systematically possible confounders that could lead to a clinical finding of an anticipation phenomenon, which may be not caused by a definite molecular-genetic mechanism. Putative confounders examined included different age structures of the parental and offspring cohorts and the possibility of an improved sensitivity in the diagnosis of IBD ("diagnostic interval").

PATIENTS AND METHODS

Study Population

In July 1996, all 8,340 affected members of the German Crohns disease and ulcerative colitis foundation (Deutsche Morbus Crohn und Colitis ulcerosa Vereinigung, DCCV e.V.) were contacted by letter. Eligible for study participation were patients with inflammatory bowel disease as documented by standard diagnostic criteria. Moreover, at least two first-degree relatives, preferably both parents (healthy or not), should have been available for molecular and epidemiological characterization. All responders completed a multi-item questionnaire together with their physicians. A dedicated telephone contact was available for patients and physicians at the Charité University Hospital in Berlin, Germany. Of 8,340 patients contacted by mail in July 1996, 2,330 responses were received by December 1996 (response rate 28%). From a representative, systematic telephone contact with 300 patients we estimated the non-response rate because of first-degree relatives not being available at 43% and the non-response rate for other reasons at 29%. The 2,330 responses yielded contacts with 2,592 patients, because of further multiple affected patients in families that were recruited through the contact with the index patient. One hundred thirteen patients were excluded as detailed below and in Table I. All patients gave informed consent before enrollment in the study. All patients were Caucasians of German extraction. The protocol was approved by the institutional ethics committee and by the regional government oversight committee on data protection ("Landesdatenschutzbeauftragter").

Clinical and Epidemiological Information

The information on the factors investigated in this study was obtained through specific items in the questionnaire: 1) the age at first symptoms and the age at diagnosis and 2) the number, age, and sex of affected relatives. Clinical questions addressed items, that could be reliably completed by the family doctor or the patient himself: 3) type of disease (Crohns disease/ulcerative colitis/undetermined colitis); 4) confirmatory diagnostic method used (endoscopy/histology/

TABLE I. Overview of the Investigated Sample[†]

Parameters	Familial cases: Affected sibling	Familial cases: Affected child	Familial cases: Affected parent	Sporadic cases
Crohn's disease (*N*)	179	64	42	1,241
Ulcerative colitis (*N*)	62	29	31	545
Indeterminate colitis (*N*)	29	10	26	221
Total (*N*)	270	103	99	2,007
Age (mean, standard deviation)	36.0 (±10.0)	31.0 (±9.0)	58.0 (±11.0)	37.5 (±11.6)
Sex (% male)	37.0	36.0	38.4	39.1

[†]2,592 patients entered the investigation. 113 patients were excluded because of failure to fill in a second confirmatory questionnaire, which was sent out 6 to 9 months later ($N = 78$) or inconsistent or incomplete data ($N = 35$). The number of analyzed individuals after exclusions in each category is given in the table. "Affected child" refers to children of affected individuals. Familial cases are only listed once, with the categories of "Affected parent" and "Affected child" taking precedence over the "Affected sibling" category.

radiology); 5) presence of stenoses, fistulae, extraintestinal manifestations (yes/no); 6) cumulative duration of hospital inpatient stays (never, less than a month, 1–3 months, 3–6 months, >6 months); 7) cumulative post-diagnosis duration of steroid medication >10 mg of prednisolon or equivalent (never, less than 1 month, 1–3 months, 3–6 months, up to 1 year, more than 1 year); and 8) total number of operations. Patients were coded as indeterminate colitis if they had changed diagnosis during the course of their disease. Interaction with the family physician or gastroenterologist was assured by the simultaneous venipuncture for a blood sample (used for DNA preparation) at the doctor's office. Questionnaires without an accompanying blood sample were not processed. In order to control the validity of the answers, all study participants received a second questionnaire repeating core items of the questionnaire in a different order and wording after an interval of 6 to 9 months. A total of 113 patients was excluded from the study because of 1) lack of completion of the second questionnaire by July 1997 ($N = 78$) and 2) differences between answers given in the original and the repeat-questionnaire and/or incomplete information ($N = 35$). Final numbers for the use of personal clinical and epidemiological data (after the described exclusions) are given in Table I.

Statistics

Statistics were performed using the SPSS software package [SPSS, 1997]. Normality of the distribution was examined with the Komolgorov-Smirnov test. The Students *t*-test and the Mann-Whitney-U test were used for comparisons of normally and non-normally distributed variables, respectively. The *P* values in Table II were corrected for multiple testing (12 tests: all tests in Table II) using the formula $P_k = 1 - (1 - P)^N$, with N denotes the number of tests.

RESULTS

Clinical Characteristics of Familial and Sporadic IBD

No difference was found in the clinical and epidemiological presentation of familial and sporadic IBD. In addition to comparisons between familial and sporadic cases, patients with a positive parental family history were compared to patients with no family history of IBD (Table II). In order to maximize the sensitivity, no corrections for multiple testing were made in the table. The marginally significant values for the number of operations ($P = 0.03$), the total duration of steroid consumption ($P = 0.03$), and the frequency of stenoses ($P = 0.05$) do not show a consistent pattern. Moreover, these differences loose significance after correction for multiple testing ($N = 12$ tests, corrected minimum $P = 0.31$).

Age of Manifestation

Manifestation of IBD was seen at a significantly younger age in children of affected individuals than in their parents (22.2 ± 0.7 (mean ± SEM) versus 41.6 ± 1.4 years, mean difference 19.4 years, $P < 0.001$). Both variables were normally distributed (Komolgorov-Smirnov test: $P = 0.6$ and 0.7). The diagnosis in the children was made a mean of 7.0 years after the parents' diagnosis was established. Children of affected parents had an earlier age at onset than individuals without a family history of IBD (22.2 ± 0.7 years versus 26.6 ± 0.2; $P < 0.001$). Age of manifestation in the fa-

TABLE II. Comparison of Familial and Sporadic IBD Cases[†]

		Familial vs. sporadic cases			Individuals with parental family history vs. None		
		Familial	Sporadic	*P*	Child of affected	No history	*P*
Hospitalizations (duration categories)	IBD	1–3 mo.	1–3 mo.	0.23*	1–3 mo	1–3 mo.	0.10*
	CD	1–3 mo.	1–3 mo.	0.75	1–3 mo.	1–3 mo.	0.82
	UC	<1 mo.	<1 mo.	0.31	<1 mo.	<1 mo.	0.37
Total duration of steroid medication (months)	IBD	4.2	4.5	0.03**	4.2	4.5	0.14**
	CD	4.4	4.7	0.14	3.6	4.3	0.33
	UC	4.1	3.8	0.11	3.1	4.0	0.12
Total number of operations (mean)	IBD	1.4	1.4	0.74**	1.0	1.4	0.03**
	CD	2.0	2.0	0.85	1.6	1.9	0.03
	UC	0.1	0.3	0.04	0.15	0.0	0.06
Stenoses (frequency)	CD	49%	44%	0.05***	46%	44%	0.69***
Fistulae (frequency)	CD	37%	40%	0.23***	31%	40%	0.07***
Extraintestinal manifestation (frequency)	IBD	71%	70%	0.67***	70%	71%	0.82***
	CD	74%	75%	0.86	73%	64%	0.31
	UC	62%	64%	0.69	63%	50%	0.28

[†]Results are not corrected for multiple testing in order to maximize the sensitivity of an explorative analysis. The comparisons were also performed in the subgroups of Crohns disease and ulcerative colitis. No significant differences were detected (data not shown).
*Median quoted for a categorical variable and *P* values from the Mann-Whitney U-test.
**Means quoted for a numerical variable. Mann-Whitney U-test used because of non-normality of the variable.
***Frequencies and *P* values from the χ^2-test (1df) reported.

milial cases (24.3 ± 0.45 years) was lower than in sporadic cases with IBD (26.6 ± 0.24 years, difference 2.3 years, $P < 0.001$).

Age Distribution of the Cohorts

The age distributions of the cohorts were significantly different. Mean ages of affected children were 31 ± 0.9 years but 58 ± 1.1 years in the affected parents, 36.0 ± 0.5 years in the familial cases, and 37.5 ± 0.8 years in the sporadic cases ($P < 0.01$ for both pair-wise comparisons, mean ± SEM).

The distribution of age-of-onset and its relationship to patient age as represented by the year of birth is shown in Figure 1. This figure demonstrates that in the younger cohort of children, observations of manifestation later in life are missing, because they will occur in the future. Consequently, the individuals in this cohort that will continue to develop IBD at a later age will are still considered unaffected and account for the ascertainment bias.

Age-Corrected Analysis

In order to account for age differences between cohorts, an age-corrected general linear model analysis was established (Fig. 2A) [SPSS, 1997]. The existence of a non-confounded anticipation effect would result in a parallel shift between the two curves in Figure 2A. However, no significant difference in the age of manifestation is seen between children of affected parents and the individuals without a family history of IBD after age correction (Fig. 2A, $P = 0.52$). Similar results are obtained when age correction is introduced into the comparison of affected parents and affected offspring (Fig. 2B, $P = 0.73$).

The diagnostic interval (time from first symptoms to diagnosis as reported by the patient) was shorter in the affected children than in their affected parents (2.4 ± 0.4 versus 4.1 ± 0.7, $P = 0.03$), thereby further decreasing the manifestation age. The diagnostic interval was age-dependent with younger patients being diagnosed earlier than their parents (general linear model, $P < 0.001$).

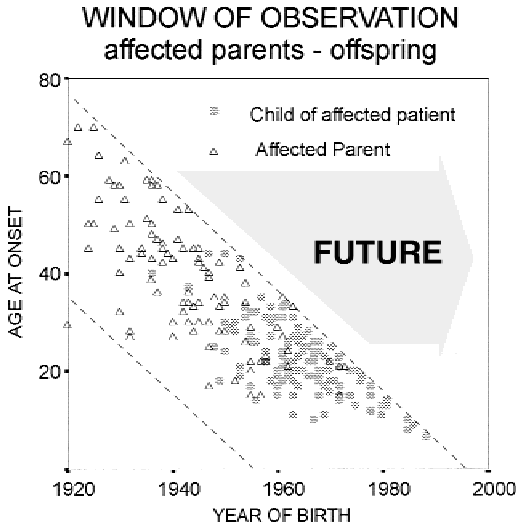

Fig. 1. The age structures and age of manifestation of the cohorts of affected parents (open triangles) and of affected offspring (gray squares). The right oblique dashed line marks the limit of observation to the future, crossing the x-axis in 1997. Most interestingly, none but one patient was diagnosed before 1955, which may be due to the specific post World War II situation in Germany and confirms the rapid rise in incidences reported at that time. The left oblique dashed line indicates this limit in the "observation window."

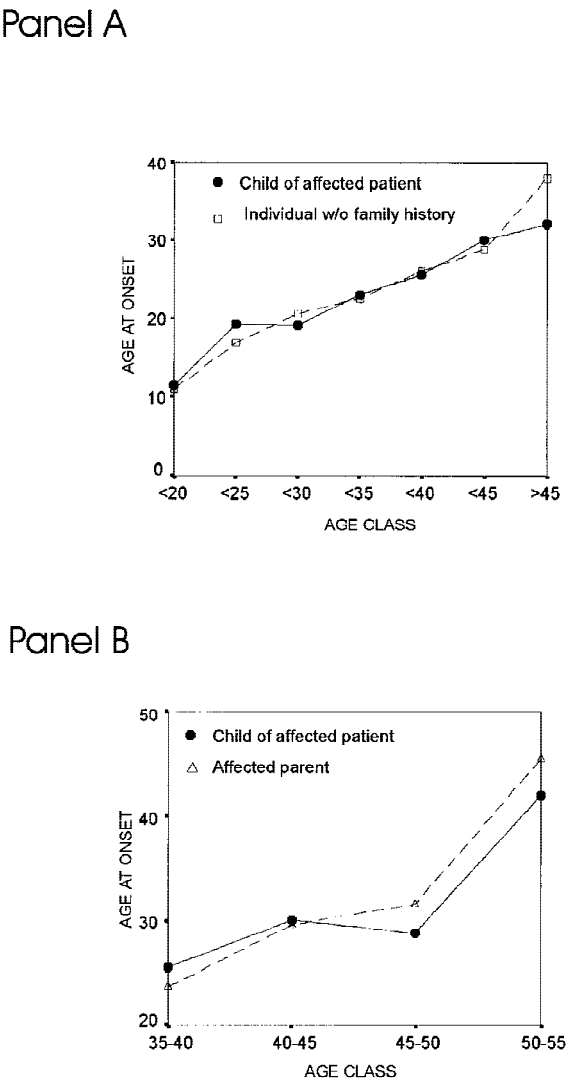

Fig. 2. Onset of disease after correction for age. **A:** Manifestation age was compared between the age corrected cohorts of patients without a parental family history of IBD and patients from multiplex families (affected parents). As evident by the overlapping curves, there is no difference in the ages of onset between the two groups after age correction in a general linear model ($P = 0.52$). **B:** A similar comparison of the cohorts of affected parents and affected offsprings with IBD. The general linear model was constructed for the age groups from 35 to 55 years, because affected parents were under-represented in the younger age groups. No difference in the age-of-onset after correction for the different age structures is seen ($P = 0.73$).

DISCUSSION

In this study, we revisited the evidence for genetic anticipation in IBD in a large German sample of familial and sporadic cases with inflammatory bowel disease. Genetic anticipation would be an attractive hypothesis, which would offer an elegant approach to disease causation. The study presented shows that children of affected parents have the same natural history of disease as children from unaffected parents. In particular, indicators of disease severity were similar among both cohorts.

The presence of a family history changes the overall risk to develop IBD as shown in other studies [Orholm et al., 1991; Satsangi et al., 1997]. However, the age-of-onset is not different, if the data set is corrected for the differences in age structure due to the generational differences in the parental and offspring groups. The raw, uncorrected age difference in our analysis confirms previously published studies, some of which had interpreted this finding as evidence of genetic anticipation [Grandbastien et al., 1998; Polito et al., 1996; Satsangi et al., 1996a, 1998]. In previous studies, the concept of genetic anticipation was supported by the finding of an expansion of unstable trinucleotide repeats in patients with inflammatory bowel disease [Cho et al., 1997]. The mechanism of trinucleotide expansion is poorly understood, although disease genes with unstable trinucleotide repeats play a definite role in some neurological disorders [Ashley and Warren, 1995; Duyao et al., 1993; The Huntingtons Disease Collaborative Research Group, 1993]. Recent studies suggest, that the expansion of this class of repeats may also be a non-specific event that occurs in the normal population [Nakamoto et al., 1997].

We think an ascertainment bias is the most likely explanation of the clinical finding of anticipation in the raw data set of IBD patients. The power to detect true anticipation in a complex disorder is quite low [Vieland and Huang, 1998]. This study strongly adds to a growing body of evidence that genetic anticipation in inflammatory bowel disease does not exist. Further prospective investigations of the natural history of inflammatory bowel disease in large long-term cohorts are necessary to confirm and extent these findings in different cohorts.

ACKNOWLEDGMENTS

The authors thank the patients and colleagues who made this study possible through their cooperation as well as Merckle GmbH and MFG gGmbH for organizational and financial support. The cooperation of the German Crohns disease and ulcerative colitis foundation (DCCV e. V.) is gratefully acknowledged. The authors thank the following additional centers in Germany for help and contributions: Dr. Theuer, Heilbronn; Dr. Meckler, Gedern; Dr. Herchenbach, Recklinghausen; Prof. Scheurlen Würzburg; Dr. Demharter, Augsburg; Dr. Simon, München; Dr. Purrmann, Moers; Dr. Jessen, Kiel; Dr. Zehnter, Dortmund; Dr. Lübke and Dr. Weismüller, Koblenz; Dr. Eiche, Denkendorf; Dr. Schönfelder Aachen; Prof. Fleig, Halle. This work was supported by public grants from the "Deutsche Forschungsgemeinschaft" (DFG: Schr 512/4-1 and SFB 415), a "Kompetenznetzwerk" grant from the BmBF (01 GI9984), and a Training and Mobility of Researchers Grant from the European Commission (ERB-4061-P2-97-0384) and MFG.

REFERENCES

Ashley CT Jr, Warren ST. 1995. Trinucleotide repeat expansion and human disease. Annu Rev Genet 29:703–728.

Cho J, Fu Y, Pickles M, Kirschner B, Hanauer SB. 1997. CAG repeat exansions in subsets of families with Crohn's disease. Gastroenterology 112:A948.

Cho JH, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR. 1998. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. Proc Natl Acad Sci 95: 7502–7507.

Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, Folstein S, et al. 1993. Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet 4:387–392.

Grandbastien B, Peeters M, Franchimont D, Gower-Rousseau C, Speckel D, Rutgeerts P, Belaiche J, Cortot A, Vlietinck R, Colombel JF. 1998. Anticipation in familial Crohn's disease. Gut 42:170–174.

Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJS, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nurnberg P, Mirza MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. 1999. A genome-wide analysis provides evidence for novel linkages in Inflammatory Bowel Disease in a large European cohort. Am J Hum Genet 64:808–816.

Hugot JP, Laurent Puig P, Gower Rousseau C, Caillat Zucman S, Beaugerie L, Dupas JL, Van Gossum A, Bonait-Pellie C, Cortot A, Thomas G. 1994. Linkage analyses of chromosome 6 loci, including HLA, in familial aggregations of Crohn disease. Am J Med Genet 52: 207–213.

Küster W, Pascoe L, Purrmann J, Funk S, Majewski F. 1989. The genetics of Crohn disease: complex segregation analysis of a family study with 265 patients with Crohn disease and 5,387 relatives. Am J Med Genet 32:105–108.

Lee JC, Lennard Jones JE. 1996. Inflammatory bowel disease in 67 families each with three or more affected first-degree relatives. Gastroenterology 111:587–596.

McInnis MG. 1996. Anticipation: an old idea in new genes. Am J Hum Genet 59:973–979.

Nakamoto M, Takebayashi H, Kawaguchi Y, Narumiya S, Taniwaki M, Nakamura Y, Ishikawa Y, Akiguchi I, Kimura J, Kakizuka A. 1997. A CAG/CTG expansion in the normal pupolation. Nat Genet 17:385–386.

Orholm M, Iselius L, Sorensen TI, Munkholm P, Langholz E, Binder V. 1993. Investigation of inheritance of chronic inflammatory bowel diseases by complex segregation analysis. BMJ 306:20–24.

Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen IA, Binder V. 1991. Familial occurrence of inflammatory bowel disease. N Engl J Med 324:84–88.

Penrose LS. 1948. The problem of anticipation in pedigrees of diastrophia myotonica. Ann Eugen 14:125–132.

Podolsky DK. 1991. Inflammatory bowel disease. N Engl J Med 325:928–937.

Polito JM 2nd, Rees RC, Childs B, Mendeloff AI, Harris ML, Bayless TM. 1996. Preliminary evidence for genetic anticipation in Crohn's disease. Lancet 347:798–800.

Satsangi J, Grootscholten C, Holt H, Jewell DP. 1996a. Clinical patterns of familial inflammatory bowel disease. Gut 38:738–741.

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. 1996b. Two stage genome-wide

search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 14:199–202.

Satsangi J, Jewell DP, Bell JI. 1997. The genetics of inflammatory bowel disease. Gut 40:572–574.

Satsangi J, Parkes M, Jewell DP, Bell JI. 1998. Genetics of inflammatory bowel disease. Clin Sci (Colch) 94:473–478.

SPSS. 1997. SPSS Statistical Algorithms. SPSS Inc., Chicago, IL.

The Huntingtons Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983.

Vieland VJ, Hodge SE. 1995. Inherent intractability of the ascerteinment problem for pedigree data: a general likelyhood framework. Am J Hum Genet 56:33–43.

Vieland VJ, Huang J. 1998. Statistical evaluation of age-at-onset anticipation: a new test and evaluation of its behaviour in realistic applications. Am J Hum Genet 62:1212–1227.